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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference **IMA/BP6015606**	**For Further Action**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International Application No. **PCT/GB_92/01892**	International Filing Date *(day/month/year)* **15 October 1992**	Priority Date *(day/month/year)* **15 October 1991**
International Patent Classification (IPC) **G01N 33/543, 33/58 C12Q 1/68**		
Applicant **MULTILYTE LIMITED ET AL**		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This **REPORT** consists of a total of **4** sheets.

   ☒ This report is also accompanied by **ANNEXES** i.e., sheets of the description, claims and/or drawings amended during international preliminary examination and/or containing rectifications made before this Authority.

   These annexes consist of a total of 8 sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

Date of submission of the demand **13 May 1993**	Date of completion of this report **7 January 1994**
Name and mailing address of the IPEA **The Patent Office, Cardiff Road, NEWPORT, Gwent, NP9 1RH**	Authorized Officer **Dr N R Curtis**
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International Application No
PCT/GB_92/01892

I. Basis of the report

1. This report has been drawn on the basis of:

☐ the international application as originally filed.

☒ the description, pages 1-35, as originally filed,
pages, filed with the demand,
pages, filed with the letter of
pages, filed with the letter of

☒ the claims, pages, as originally filed,
pages, as amended under Article 19,
pages, filed with the demand,
pages 35-42, filed with the letter of 6 December 1993
pages, filed with the letter of

☒ the drawings, sheets 1-8, as originally filed,
sheets, filed with the demand,
sheets, filed with the letter of
sheets, filed with the letter of

2. The amendments have resulted in the cancellation of: pages:
sheets of drawings No:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box.

4. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

☐ copy of the earlier application whose priority has been claimed.

☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	claims 1-25	YES
	claims	NO
Inventive Step (IS)	claims 1-25	YES
	claims	NO
Industrial Applicability (IA)	claims 1-25	YES
	claims	NO

2. CITATIONS AND EXPLANATIONS

All claims meet the requirement of industrial applicability.

Claims 1-25 meet the requirements of both novelty and inventive step because there is no disclosure nor suggestion in the documents cited in the International search report of a binding assay or kit which employs microspheres and capture binding agent which is immobilised at high density to a solid support. Moreover, the advantages displayed by such an assay or kit could not have been predicted from an assay or kit which combined the use of microspheres and capture binding agent immobilised at high density to a solid support which features have been disclosed separately in the prior art. (See for example WO 89/01157, EP 267317).

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

It is considered that the use of the term "high density" as used in Claims 1, 19 and 22 renders the scope of these claims unclear. With reference to this matter, the description at page 33, lines 30-33 details that the capture binding agent is confined to a small number, bound at high surface density to a very small area in the form of microspots. However, Claims 1, 19 and 22 which detail "..., the capture agent being immobilised at high density ..." does not adequately define such "microspots" which would appear to be essential for the assay and kit of the invention.

The statements of invention are inconsistent with independent Claims 1, 19 and 22.

It is noted that the microspheres of Claims 1 and 19 have a size of less than 5μm. However, there is no size restriction on the microspheres detailed in Claim 22.

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CLAIMS

1. A binding assay process in which the concentration of an analyte in a liquid sample is determined by comparison with a dose-response curve computed from standard samples,

using a capture binding agent having binding sites specific for the analyte and a developing binding material capable of binding with the bound analyte or with the binding sites on the capture binding agent occupied by the bound analyte or with the binding sites remaining unoccupied on the capture binding agent,

the capture binding agent being used in an amount such that only an insignificant fraction of the analyte in the sample becomes bound to the capture binding agent, and

a label being used in the assay in relation to the developing binding material whereby the strength of the signal associated with the label is representative of the fractional occupancy of the binding sites on the capture binding agent by the analyte,

wherein there is used as the label microspheres having a size of less than 5 μm and carrying a marker.

2. A process as claimed in claim 1, wherein the microspheres have a uniform size of 0.01 to 0.5 μm.

3. A process as claimed in claim 1 or 2, wherein the microspheres are made of polymer latex and are provided on their surface with negatively charged or positively charged groups.

4. A process as claimed in any of claims 1 to 3, wherein the marker is a fluorescent label contained within the microspheres.

5. A process as claimed in claim 4, wherein the microspheres contain molecules of an oil-soluble fluorescent dye providing fluorescence in a colour range compatible with

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a standard filter set.

6. A process as claimed in claim 4, wherein the microspheres contain molecules of a fluorescent dye having a prolonged fluorescent period such that the signal strength is capable of being determined by a time-resolved fluorescence technique.

7. A process as claimed in any of claims 1 to 6, wherein the microspheres have the developing binding material adsorbed or directly or indirectly chemically bonded to them.

8. A process as claimed in any of claims 1 to 6, wherein some of the microspheres have the developing binding material adsorbed or directly or indirectly chemically bonded to them and others of the microspheres have the capture binding agent adsorbed or directly or indirectly chemically bonded to them, the label contained in the microspheres to which the developing binding material is adsorbed or chemically bonded being different from the label in the microspheres to which the capture binding agent is adsorbed or chemically bonded.

9. A process as claimed in claim 7 or 8, wherein, after the developing binding material and where appropriate the capture binding agent have been linked to the microspheres by adsorption or covalent bonding, the microspheres are blocked to avoid their non-specific binding to other materials.

10. A process as claimed in claim 9, wherein the blocking of the microspheres is achieved by means of bovine serum albumin or other non-interfering protein material and a non-ionic detergent.

11. A process as claimed in any of claims 1 to 10,

wherein the capture binding agent is immobilised on a solid support in the form of one or more microspots having an area of 1 mm^2 or less at a surface density in the range of 1,000 to 100,000 molecules/ μm^2, and wherein the liquid sample size is 1 ml or less.

12. A process as claimed in claim 11, wherein the microspot or microspots have a diameter of 0.01 to 1 mm and contain immobilised capture binding agent at a surface density of 10,000 to 50,000 molecules/ μm^2, the sample size being 50 μl - 1 ml.

13. A process as claimed in claim 11 or 12, wherein different capture binding agents are immobilised on different microspots on the same solid support and different binding assays for the determination of different analytes in the same liquid sample are performed in the same operation.

14. A process as claimed in any of claims 1 to 13, wherein both the capture binding agent and the developing binding material are antibodies.

15. A process as claimed in any of claims 1 to 13, for use in DNA assays, wherein the capture binding agent is a single-stranded oligonucleotide DNA probe recognising a corresponding DNA sequence in the liquid sample and the developing binding material either is an antibody recognising only twin-stranded DNA sequences or is an oligonucleotide DNA sequence which either recognises another part of the corresponding DNA sequence in the liquid sample or recognises residual single-stranded oligonucleotide DNA probe forming the capture binding agent, the developing binding material being labelled by means of the microspheres.

16. A process as claimed in any of claims 1 to 15, wherein the binding assay is a non-competitive binding

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assay.

17. A binding assay process for the detection of an analyte comprising a single-stranded DNA sequence in a liquid sample, comprising

contacting the sample in a non-competitive or competitive procedure with

an immobilised capture binding agent which is a single-stranded oligonucleotide DNA probe capable of recognising analyte in the liquid sample and binding therewith, and

with a labelled developing binding material which either is an antibody capable of recognising only twin-stranded DNA sequences formed from the probe and the analyte and of binding therewith or is an oligonucleotide DNA sequence capable of recognising and binding with either another part of the analyte or the residual probe,

the developing binding material being labelled by means of microspheres having a size of less than 5 μm and carrying a marker, and,

after the removal of unattached developing binding material, detecting the presence of the analyte by the existence or strength of a signal from the marker attached to developing binding material which has become bonded directly or indirectly to the immobilised capture binding agent.

18. A process as claimed in claim 17, wherein the marker is a fluorescent label contained within microspheres having a size of 0.01 to 1 μm.

19. A process as claimed in claim 17 or 18, wherein the developing binding material is directly or indirectly covalently bonded to the microspheres.

20. A kit for use in a binding assay process in which the concentration of an analyte in a liquid sample is determined using a capture binding agent having binding

sites specific for the analyte and a developing binding material capable of binding with the bound analyte or with the binding sites on the capture binding agent occupied by the bound analyte or with the binding sites remaining unoccupied on the capture binding agent, a label being used in relation to the developing binding material whereby the strength of the signal associated with the label is representative of the fractional occupancy of the binding sites on the capture binding agent by the analyte, the kit comprising (a) a solid support having the capture binding agent immobilised thereon, (b) a developing reagent comprising the developing binding material adsorbed or directly or indirectly chemically bonded to the surface of microspheres carrying a marker and (c) standards having known amounts or concentrations of the analyte to be determined.

21. A kit as claimed in claim 20, wherein the reagent contains the developing binding material adsorbed onto or covalently bonded to microspheres having a size of less than 5 μm and containing molecules of a fluorescent dye.

22. A kit as claimed in claim 20 or 21, wherein the solid support has the capture binding agent immobilised thereon in the form of one or more microspots of size less than 1 mm^2 and surface density at least 1000 molecules/ μm^2.

23. A kit as claimed in claim 22, wherein different capture binding agents are immobilised on different microspots on the same solid support and a plurality of different developing reagents and different standards for different analytes are included.